REMARKS

In view of the above amendments and the following remarks, reconsideration of the outstanding office action is respectfully requested.

Pseudomonas syringae pv. tomato DC3000 is a widely studied model plant pathogen that causes disease on tomato and *Arabidopsis*. DC3000 uses a type III secretion (TTSS) system to directly deliver bacterial effector proteins into the host cell. Loss of function mutations in the TTSS completely abrogate *P. syringae* disease formation, indicating that effectors are essential agents of *P. syringae* pathogenesis. In bacterial pathogens of plants, the TTSS is encoded by the hypersensitive response ("HR") and pathogenicity (*hrp*) genes. Mutations in key *hrp* genes prevent the secretion of effectors and inhibit pathogen growth and host defenses. A hallmark of effector genes is the presence of a "Hrp box" cis element in their promoter which is recognized by the HrpL ECF-like sigma factor. A recent search for Hrp box containing genes in the genome of *Pseudomonas syringae pv. tomato* strain DC3000 revealed over 20 putative effector genes. Although the role of effector proteins in pathogen virulence is poorly understood, many effectors have been isolated based on their ability to trigger host immunity.

In the "gene-for-gene" model of plant immunity, disease resistance is initiated by recognition of a pathogen avirulence (Avr) effector protein by a plant resistance (R) protein. The tomato R protein Pto, a serine/threonine protein kinase, recognizes and directly interacts with DC3000 effector proteins AvrPto and AvrPtoB, and initiates immunity in tomato by characterized and uncharacterized signaling mechanisms. Interestingly, the Pto kinase shares sequence similarity with the human interleukin-1 receptor associated kinase (IRAK) and with the *Drosophila* Pelle kinase, both of which, like Pto, play a role in immune responses. The *Pto* gene belongs to a gene family of 6 members on tomato chromosome 5. One of these family members, *Fen*, encodes a kinase that confers sensitivity to an insecticide (fenthion), while the function of the others is unknown.

The R gene-mediated plant immune response is characterized by a series of physiological changes in the plant cell, including the formation of reactive oxygen species, induction of defense genes, and the HR. The HR is defined as a defense response involving rapid, localized cell death that functions to limit pathogen growth. The cell death associated with the HR is a genetically controlled and regulated process and an example of programmed cell death in plants. As such, programmed cell death is a hallmark of HR-based immunity in

plants, and cell death phenotypes are often used in laboratory experiments to discover and dissect plant immune responses.

The AvrPtoB protein has a predicted molecular mass of 59 kDa, is secreted via the TTSS, and triggers the HR and immunity in Pto-expressing tomato plants. AvrPtoB has limited similarity to AvrPto; however, it shares 52% amino acid identity with the *P. s. pv. phaseolicola* effector VirPphA. In general, bacterial effector proteins are highly diverse with little amino acid sequence similarity among them (one exception is the AvrBs3 family). They have been identified from all four of the most common genera of plant bacterial pathogens (i.e., *Pseudomonas, Xanthomonas, Erwinia, and Raistonia*). In a still cryptic process, these pathogens utilize the TTSS to inject effectors across the plant cell wall into the cytoplasm. Little is known of the fate of bacterial effectors once they are in the plant cell although some members of the AvrBs3 family are localized to the nucleus, some effector proteins are targeted to the plasma membrane after being myristylated, and others are processed to smaller forms.

The AvrPto protein and the Pto kinase physically interact in a yeast two-hybrid system. Co-expression of Pto and AvrPto as transgenes in a *pto* mutant leaf is sufficient to activate resistance. Mutations that disrupt this interaction also abolish the ability to elicit disease resistance in plant leaves. Resistance is dependent on the Prf protein which bears striking similarity to the large NB-LRR class of R proteins. Pto-Fen chimeras were used to define the kinase activation loop as a key determinant of Pto interaction specificity for AvrPto. Pto kinase is phosphorylated on 8 residues and mutation of two of these residues (T38 and S198) abolishes its ability to elicit host resistance. Recognition specificity of Pto for AvrPto appears to have evolved before *Lycopersicon* speciation because a Pto family member from a distantly related species, *L.hirsutum*, also recognizes AvrPto.

The AvrPto gene was originally isolated from *P. s. tomato* strain JL1065 based on its ability to confer avirulence to a virulent strain of *P. s.maculicola*. AvrPto encodes an 18 kD protein that bears little sequence similarity to proteins in current databases. Its mechanism of activating resistance is unknown although it likely interacts with Pto inside the plant cell and possibly with certain 'AvrPto-dependent Pto-interacting' (Adi) proteins as part of a complex. AvrPto acts as a virulence factor when Pto (or Prf) is absent from the plant cell and increases the growth of *P. s. tomato* about 10-fold as compared to a strain lacking the effector. In common with several effectors, AvrPto has a myristylation motif at its N terminus that is required for both its avirulence and virulence activity. The amino acids of

AvrPto that are required for its recognition by the Pto kinase have been examined by saturation mutagenesis. Mutation of three AvrPto residues -- S94, I96, and G99 -- abolishes interaction with Pto and avirulence activity, but not virulence activity, in tomato. Along with the other observations, these results indicate that an internal region of AvrPto determines its binding specificity for Pto.

AvrPto-like DNA sequences are present in *Pseudomonas* strains that are known to be avirulent on *Pto* tomato plants (race 0 strains) and are absent from virulent ones (race 1 strains). Thus, a homolog of *avrPto* was identified in avirulent *P. s. tomato* strain DC3000 based on DNA blot hybridization. Gene replacement strains in which the *avrPto* reading frame was deleted were constructed in strains JL1065 and DC3000. Surprisingly, both mutant strains were still recognized by *Pto*-expressing tomato leaves. A later study found that a tomato line carrying a CaMV 35S::*Pto* transgene (and not a sibling line without *Pto*) is resistant to the *avr*PtoΔDC3000 deletion strain. These results implied that strains DC3000 and JL1 065 carry additional avirulence proteins that are recognized specifically by Pto.

In recent years, evidence has accumulated that effector proteins can interfere with host defense responses. In a breakthrough study, it was demonstrated that VirPphA allows *P. s. pv. phaseolicola* to evade HR-based immunity in bean. Other *P. s. pv. phaseolicola* effectors also allow the pathogen to avoid triggering host immunity, including AvrPphC and AvrPphF. Additionally, in the *P. s. pv. Maculicola*-Arabidopsis pathosystem, interference has been observed with the effector proteins AvrRpt2 and AvrRpml and the HR initiated by the R proteins RPS2 and RPM1, respectively. These findings suggest that for some effector proteins virulence activity can be dominant over avirulence activity. Although the phenomenon of effector-mediated evasion of plant immunity has been well documented, the molecular basis of this activity has remained mysterious. Several hypotheses have been proposed to explain how some effector proteins (such as VirPphA, AvrPphC and AvrPphF) prevent a host from detecting a pathogen, including: i) inhibition of *avr* gene expression; ii) blocking of Avr protein secretion or translocation; iii) interference with Avr/R protein recognition inside the plant cell; or iv) suppression of HR or disease resistance signaling downstream of Avr recognition. Specific support, however, for any one of these hypotheses has not been reported.

The present invention is directed to overcoming these and other deficiencies in the art.

The rejection of claims 1-6 under 35 U.S.C. § 101 as being directed to non-statutory subject matter is respectfully traversed in view of the above amendments cancelling the rejected claims.

The rejection of claims 1-6 and 103-109 under 35 U.S.C. § 112 (1st para.) for lack of enablement is respectfully traversed.

The U.S. Patent and Trademark Office ("PTO") has taken the position that the specification provides enablement for the inhibition of programmed cell death in eukaryotes. Since the claims are now commensurate in scope with what the PTO has deemed to be enabled, the rejection under 35 U.S.C. § 112 (1st para.) should be withdrawn.

The rejection of claim 1 under 35 U.S.C. § 102(b) as being anticipated by Mangan et al., "Stimulation of Human Monocytes by Endotoxin-Associated Protein: Inhibition of Programmed Cell Death (Apoptosis) and Potential Significance in Adjuvanticity," *Infect Immun* 60(4):1684-1686 (1992) ("Mangan") is respectfully traversed in view of the above amendments cancelling the rejected claim.

The rejection of claims 1-6 under 35 U.S.C. § 102(a) as being anticipated by either Kim et al., "Two Distinct *Pseudomonas* Effector Proteins Interact with the Pto Kinase and Activate Plant Immunity," *Cell* 109:589-598 (2002) ("Kim") or Fouts et al., "Genomewide Identification of *Pseudomonas syringae* pv. *Tomato* DC3000 Promoters Controlled by the HrpL Alternative Sigma Factor," *PNAS* 99(4):2275-2280 (2002) ("Fouts") is respectfully traversed in view of the above amendments cancelling the rejected claims.

In view of all of the foregoing, it is submitted that this case is in condition for allowance and such allowance is earnestly solicited.

Respectfully submitted,

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